

Yoshihiko SATÔ* & Sumi INOUE**: **Embryo sac formation
of *Deutzia crenata forma bicolor***

佐藤嘉彦*・井上珠実**: サラサウツギの胚囊形成

The genus *Deutzia* has been assigned variously to the Saxifragaceae by Engler (1891) and Schulze-Menz (1964), to the Hydrangeaceae by Cronquist (1968, 1981), and to the Philadelphaceae by Hutchinson (1959) and Airy Shaw (1973). However, *Deutzia* has been treated as a genus which has a near relation to the genus *Philadelphus* by all of these taxonomists. Unfortunately, these two genera have not received much attention from an embryological point of view. Satô & Inoue (1985) have just made a report on the ovular structure and the embryo sac formation in *Deutzia scabra*, *D. crenata* and *Philadelphus satsumi*. These three species have an embryologically prominent feature. An embryo sac of these species is formed according to the monosporic eight-nucleate *Polygonum* type of development. In the process of embryo sac formation, a four-nucleate embryo sac elongates greatly to pass through a micropylar canal, so that the micropylar portion of the embryo sac goes out into an ovarian locule. When the embryo sac matures, in these two species of *Deutzia* the size of extra-micropylar portion attains to about half the size of the whole embryo sac. In *P. satsumi*, only the micropylar apex of the mature embryo sac protrudes into the ovarian locule. Thus, the occurrence of the extra-micropylar embryo sac in these two genera, *Deutzia* and *Philadelphus*, can be regarded as one of the features which represent a close affinity between them (Satô & Inoue 1985), because it is in very restricted genera among the angiospermous plants that the extra-micropylar embryo sac occurs (Maheshwari 1950, Johri 1963).

In *Vandellia* and *Torenia* of the Scrophulariaceae, there is a series of variations from extra-micropylar embryo sac to intra-micropylar embryo sac (Yamazaki 1955). Whether the embryological variations similar to those found

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in the Scrophulariaceae occur also in *Deutzia* and some other genera which have been suggested to have a near relation to *Deutzia* or whether they don't is interesting matter, because it seems that the information to elucidate more objectively a relation between *Deutzia* and its allied genera will be obtained. As the fact which seems to show more evidently a close affinity between *Deutzia* and *Philadelphus* was observed in the developmental process of embryo sac of *Deutzia crenata forma bicolor*, we report on it in this paper.

Material and method At appropriate intervals extending from April to June in 1982-1984, many buds and flowers of *Deutzia crenata forma bicolor* were collected from individuals planted on the campus of the Yokohama National University, Hodogaya-ku, Yokohama City. All materials collected were fixed in formalin-acetic-alcohol (FAA). After then they were dehydrated in ethyl alcohol-*tert.* butyl alcohol series and embedded in paraffin or histosec (supplied by Merck & Co., Ltd.). They were sectioned serially at 6-10 μm thick and stained with Heidenhain's iron alum hematoxylin and fast green combination.

Observation Ovary and ovule. Ovary of *Deutzia crenata forma bicolor* was inferior. It was incompletely divided into loculi, of which the number was the same as that of carpels. The number of carpels was usually four, rarely three. Many ovules developed on the parietal placentae. The ovule (Figs. 1A, 2D, H) was usually hemianatropous and was composed of a thin nucellus which was enclosed by one integument. The nucellus was composed of single axial row of cells covered by a layer of epidermal cells. The apical cell of the axial row elongated along the axis of the nucellus and functioned as a megasporocyte (Figs. 1A, B) without any divisions. When the megasporocyte began to divide meiotically, the integument had already grown beyond the level of the nucellar tip, and it had begun to form a micropylar canal (Fig. 1A). In the ovule where the meiosis was proceeding, the cells at and near the apex of the integument, especially the cells on the side opposite to the funicle, grew in the same direction as that of the growth of integument to form a "membraneous structure" at the opening of the micropylar canal (Figs. 1A, 2D). The cells, except the cells of the apical portion, of the inner epidermis of the integument grew at right angles to the surface of the integument (Figs. 1A, 2D, E) and functioned as the integumentary tapetum.

Embryo sac formation. The megasporocyte underwent two successive meiotic divisions. Each of them was always accompanied by wall formation.

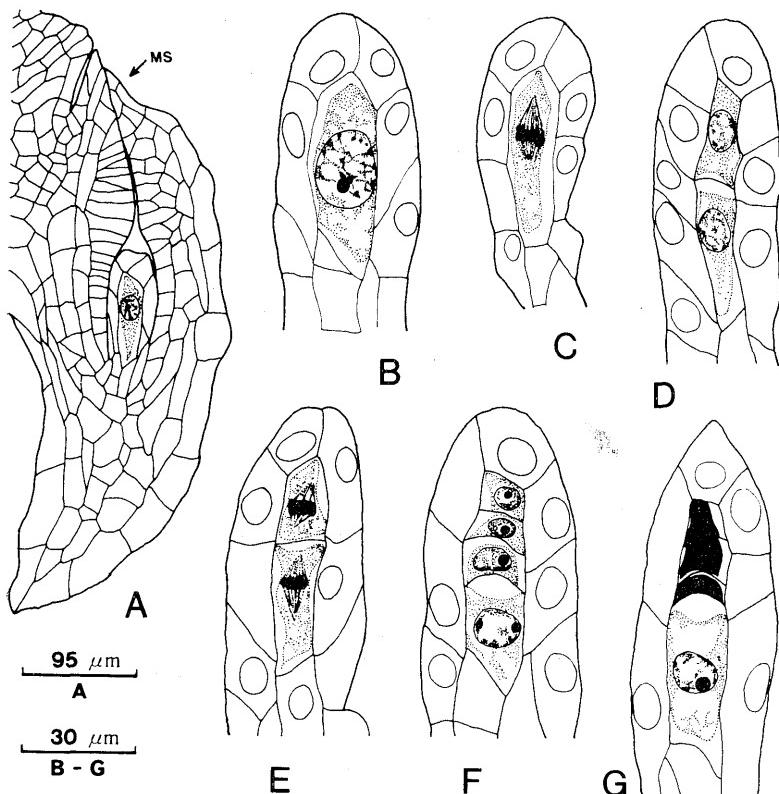


Fig. 1. Megasporogenesis of *Deutzia crenata* forma *bicolor*. A. Ovule with megasporocyte. A membranous structure begins to be formed at the opening of the micropylar canal. B. Nucellus with megasporocyte. C. Nucellus with megasporocyte in division. D. Nucellus with dyad. E. Nucellus with two dyad cells, each in division. F. Nucellus with linear tetrad. G. Nucellus with functional megasporite. MS: membranous structure.

The first meiotic division (Fig. 1C) of the megasporocyte was in the transverse plane. Two dyad cells (Fig. 1D) were produced by this division. The second division (Fig. 1E), which was also transverse, took place simultaneously in two dyad cells. As a result, a tetrad (Fig. 1F), of which four megaspores were invariably arranged in line, was produced. The micropylar three megaspores of them degenerated, while only the chalazal one (Fig. 1G) became functional and developed into an embryo sac.

The functional megasporangium underwent the first division of its nucleus (Fig. 2A) to form a two-nucleate embryo sac (Fig. 2B). The two nuclei moved apart to the opposite poles of the embryo sac and a vacuole intervened between them. As the embryo sac elongated along the axis of the nucellus, the cytoplasm gradually began to become richer around the nucleus at the micropylar end than around the nucleus at the chalazal end of the embryo sac. Usually, those two nuclei divided simultaneously to form four nuclei, arranged in pairs, at the micropylar and chalazal ends of the embryo sac (Fig. 2C). However, two kinds of three-nucleate embryo sac were observed in some ovules. One of them had two nuclei at the micropylar end and one nucleus at the chalazal end (Fig. 2E); the other had two nuclei at the chalazal end and one nucleus at the micropylar end (Fig. 2F).

The micropylar apex of the four-nucleate embryo sac elongated rapidly and greatly to intrude into the micropylar canal. Epidermal cells of the nucellus degenerated and disappeared except a few epidermal cells at the base of nucellus. The micropylar apex of the embryo sac continued to elongate further and passed through the micropylar canal. It seems that the integumentary tapetum surrounding the micropylar canal has something to do with the promotion of elongation of the embryo sac. Immediately after the micropylar apex of the embryo sac went out into the ovarian locule, the embryo sac changed the course of elongation toward the point of funicle attachment. It seems that the "membraneous structure" formed at the opening of the micropylar canal to the ovarian locule has something to do with this change of the elongation course. Before the four nuclei began to divide, the elongation of the embryo sac was nearly completed and after then the embryo sac hardly became longer. There was a variation in respect to the ratio of the size of the extra-micropylar portion to the size of the whole embryo sac. A maximum ratio reached about one to three (Fig. 2G). On the other hand, there also was an embryo sac (Fig. 2H) of which only the micropylar apex protruded into the ovarian locule.

These four nuclei divided to form eight nuclei, arranged in quartets, at the micropylar and chalazal ends of the embryo sac. Three of the quartet at micropylar end of the eight-nucleate embryo sac became differentiated as an egg apparatus. The apparatus always lay on the outside of the ovule and consisted of an egg cell, which was flanked by two synergids. Three of the

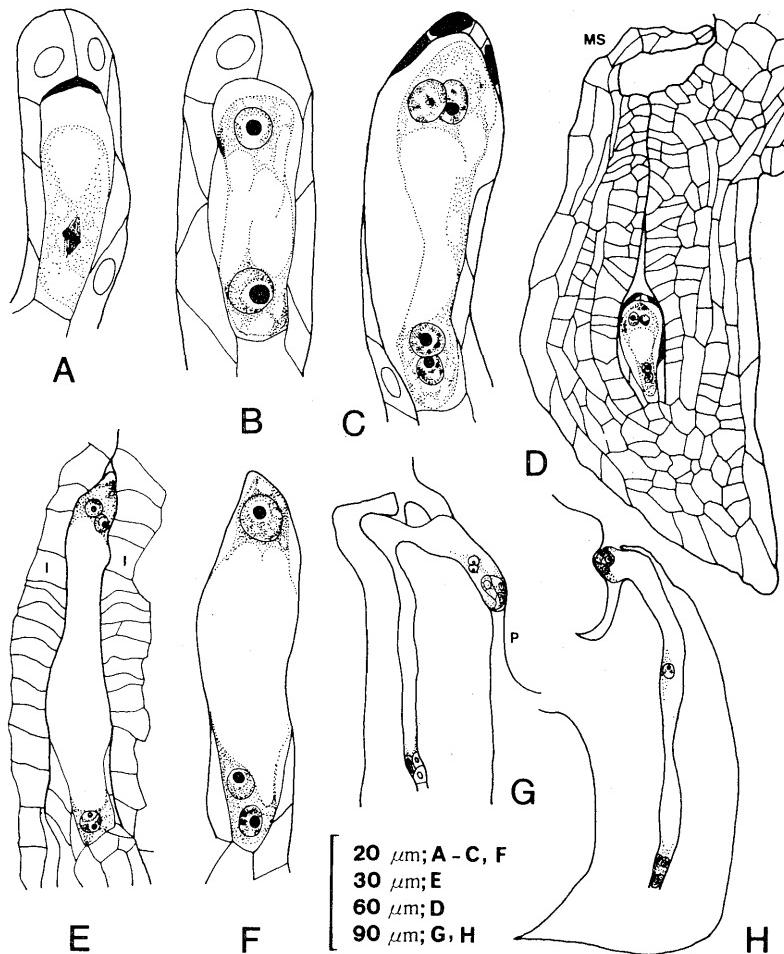


Fig. 2. Megagametogenesis of *Deutzia crenata* forma *bicolor*. A. Nucellus with functional megasporite, of which the nucleus is dividing. B. Nucellus with two-nucleate embryo sac. C. Nucellus with four-nucleate embryo sac. D. Ovule with four-nucleate embryo sac. A membranous structure is formed at the opening of the micropylar canal. E. Portion of integumentary tapetum and nucellus with three-nucleate embryo sac, which is composed of two micropylar nuclei and one chalazal nucleus. F. Nucellus with three-nucleate embryo sac, which is composed of one micropylar nucleus and two chalazal nuclei. G. Portion of ovule with mature embryo sac. The micropylar one-third of embryo sac protrudes from the micropylar canal. Two polar nuclei are about to fuse near the egg apparatus. One of the three antipodal cells is degenerating. H. Ovule with mature embryo sac. A micropylar apex of the embryo sac protrudes from the micropylar canal. The two nuclei have already fused to form a diploid nucleus. I: integumentary tapetum, MS: membranous structure, P: placental region.

quartet at the chalazal end of the embryo sac were differentiated as antipodal cells. The two remaining nuclei migrated from the opposite ends of the embryo sac and they became polar nuclei. They, usually, fused near the egg apparatus (Fig. 2G). Some antipodal apparatus (Fig. 2G) began to degenerate before the fusion of the two polar nuclei, and some (Fig. 2H) persisted after the fusion of them but they degenerated before fertilization.

Discussion In *D. crenata* forma *bicolor*, the ovule is tenui-nucellate, unitegmic and usually hemianatropous, and the embryo sac is formed according to the monosporic eight-nucleate *Polygonum* type of development. In the process of embryo sac formation, the four-nucleate embryo sac elongates greatly, so that its micropylar apex goes out into the ovarian locule through the micropylar canal. *D. crenata* forma *crenata* and *D. scabra* have an extra-micropylar embryo sac as well (Satô & Inoue 1985). Therefore, an extra-micropylar embryo sac seems to distribute widely within *Deutzia*. However, the extent of protrusion of the embryo sac to the ovarian locule is various. Usually, the size of the extra-micropylar portion is beyond half of the embryo sac in *D. crenata* forma *crenata*, though it is below half of the embryo sac in *D. scabra* (Satô & Inoue 1985). In *D. crenata* forma *bicolor* examined here, there is a series of variations from embryo sac of which only the micropylar apex protrudes from the micropylar canal to embryo sac of which a third part on the micropylar side protrudes. In *Philadelphus*, which has been considered to have a near relation to *Deutzia* by almost all taxonomists, only the micropylar apex of the embryo sac protrudes to the ovarian locule (Satô & Inoue 1985). The embryo sac of *D. crenata* forma *bicolor*, in this respect, has a feature intermediate between that of *D. crenata* forma *crenata* and *D. scabra* and that of *Philadelphus*.

Two kinds of three-nucleate embryo sac were observed in the process of embryo sac formation of *D. crenata* forma *bicolor*. One of them was composed of two micropylar nuclei and one chalazal nucleus, while the other was composed of one micropylar nucleus and two chalazal nuclei. It seems that the former was produced owing to a division of the micropylar nucleus which preceded a division of the chalazal nucleus in a two-nucleate embryo sac, while the latter was produced owing to a division of the chalazal nucleus which preceded a division of the micropylar one. In the process of embryo sac formation of *D. crenata* forma *crenata* (Satô & Inoue 1985), the phenomenon that a nucleus or nuclei at the micropylar end of the developing embryo sac divide

earlier than a nucleus or nuclei at the chalazal end is infrequently observed. In *P. satsumi* (Satô & Inoue 1985), however, the phenomenon that a nucleus or nuclei at the chalazal end divide earlier than a nucleus or nuclei at the micropylar end is frequently observed during megagametogenesis. This phenomenon has not been observed during the embryo sac formation in *Deutzia* examined hitherto. That is, both the phenomena occur during the embryo sac formation of *D. crenata* forma *bicolor* examined here.

There is an embryologically peculiar feature in the process of embryo sac formation of *Deutzia* examined hitherto and in that of *Philadelphus*, respectively. In the process of embryo sac formation of *Deutzia crenata* forma *bicolor*, however, there is not only a feature which is intermediate between that of the other members of *Deutzia* and that of *Philadelphus*, but also a feature which is found in each of the processes of embryo sac formation of these two genera. Thus, by the embryological feature of *D. crenata* forma *bicolor*, that of *Deutzia* examined hitherto is linked with that of *Philadelphus*. This seems to represent that these two genera have a near relation with each other.

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ウツギ属のサラサウツギの胚囊形成過程を調査した。胚囊は单胞子性8核タデ型に従って形成される。4核期の胚囊は著しく伸長し、珠孔を抜け、珠孔側の一部が胚珠の外に裸出する。胚囊の珠孔端だけが裸出するものから胚囊の約1/3が裸出するものまで、様々な変異がみられた。胚囊形成過程が調べられているウツギ属のマルバウツギやウツギでは、胚囊の約1/2が裸出するが、バイカウツギ属のバイカウツギでは、珠孔端だけが裸出するにすぎない (Satô & Inoue 1985)。さらにサラサウツギでは、珠孔端に2核と合点端に1核を持つ3核期の胚囊と珠孔端に1核と合点端に2核を持つ胚囊がときどき観察された。これは2核期の胚囊の2核の分裂が同調していないために生じた現象と考えられる。珠孔端の核が合点端の核よりも先に分裂する現象はウツギでみられ、合点端の核が先に分裂する現象はバイカウツギでみられている (Satô & Inoue 1985)。これまでに調査されたウツギ属とバイカウツギ属の胚囊形成過程では、それぞれ独特な特徴がみられる。サラサウツギはウツギ属とバイカウツギ属の中間的な発生学的特徴を持つとともに、両属に独特と看されてきた発生学的特徴を合せ持っている。つまり、これまでに調査されたウツギ属とバイカウツギ属の発生学的特徴は、サラサウツギのそれによって結び付けられると考えられる。このことは両属が近縁な関係にあることを示すものと考えてよいであろう。

□Szániszlo, P. J. & J. L. Harris (ed.): **Fungal dimorphism, with emphasis on fungi pathogenic for humans** 395pp. 1985. Plenum Pub. Co., New York. テキサス大学の Szániszlo 教授が Harris 教授とともに編集したもので、23名の学者の協力を得ている。一般菌類形態学、酵母の組織形態学、酵母と菌類の組織、アイソトロピック状に肥大した組織形態、2型性のケカビ類の5部門に分けて記述している。扱った菌類は動物、とくに人体寄生菌で、糸状菌の *Sporothrix*, *Ceratocystis*, *Chrysosporium*, *Candida*, *Exophiala*, *Mucor*, および酵母類である。主な問題は、自然と培養状態の諸菌について、2型性の微細構造、温度による変化、かけ合わせの反応、酵母の分裂、環境による制御、組織培養法、菌糸の成長、核融合、菌と寄主との関係、呼吸作用と醣酵、lipid 合成、酵素合成、DNA 生成と核分裂との関係、胞子の発芽、菌糸より酵母細胞への移行、炭素と窒素の代謝等である。

(小林義雄)